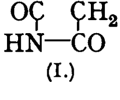
239. *Ultra-violet Absorption Spectra of Nitrogenous Heterocyclic Compounds. Part II. Effect of* p_H *and Irradiation on the Spectrum of Barbituric Acid.*

By John R. Loofbourow and Miriam M. Stimson.

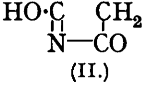
The ultra-violet absorption of barbituric acid in aqueous solution at various p_H's changed from a maximum molecular extinction of 31,000 at p_H 7·0 to 25,000 at p_H 11·0 and to a minimum of 6800 at p_H 3, the lowest p_H investigated. The marked change in absorption with change in p_H is consistent with the theory that tautomerism of the amide–imidol type is responsible for the change, since three groups in this molecule can undergo such isomerisation. The sharp peak at p_H 7·0 indicates the additive absorption of several groups with similar absorbing characteristics. Ultra-violet irradiation resulted in a rapid progressive decrease in extinction at the 2600 A. maximum, followed by increased absorption in the short-wave and the long-wave region of the spectrum.

The ultra-violet absorption spectrum of barbituric acid has been reported by several workers (Macbeth, Nunnan, and Traill, J., 1926, 1248; Marchlewski and Wiersuchowska, *Bull. Acad. Polonaise*, 1929, *A*, 66; Castille and Ruppol, *Bull. Soc. Chim. biol.*, 1928, **10**, 623; Heyroth and Loofbourow, *J. Amer. Chem. Soc.*, 1934, **56**, 1728; Fredholm, Diss., Upsala, 1937), but only for limited p_H's. As stated in Part I (this vol., p. 844), Heyroth and Loofbourow (*loc. cit.*) suggested, because of the markedly greater change of absorption with change in p_H which they found for uracil (*J. Amer. Chem. Soc.*, 1931, **54**, 3441) and for guanine (*loc. cit.*, 1934) than that reported by Holiday (*Biochem. J.*, 1930, **24**, 619) for adenine, that tautomerism of the amide–imidol type ($\text{NH·CO} \rightleftharpoons \text{N:C·OH}$) might account for the influence of p_H on the absorption of purines and pyrimidines. Although we reported in Part I (*loc. cit.*) that, contrary to Holiday's data, the spectrum of adenine does change appreciably in response to p_H change, yet this effect (attributed by us to tautomerism of the imidine type: $\text{N:C·NH}_2 \leftrightarrows \text{NH·C:NH}$) is less than that reported for uracil (Heyroth and Loofbourow, *loc. cit.*, 1931), which contains two carbonyl groups, or for guanine (Holiday, *loc. cit.*; Heyroth and Loofbourow, *loc. cit.*, 1934), which contains one. It seems probable, therefore, that amide–imidol tautomerism may account primarily for the relation of absorption to p_H in those purines and pyrimidines in which such tautomerism is possible. If this is true, barbituric acid, which contains three carbonyl groups and can theoretically undergo a large number of tautomeric rearrangements, some of which are shown in formulæ (II)—(V), should show especially marked influence of p_H on absorption. We therefore undertook a study of the absorption of this acid over a wide range of p_H. The results show that the change of absorption of this compound with change in p_H is more marked than that hitherto reported for any other purine or pyrimidine, and thus substantiate the hypothesis with regard to the influence on absorption of tautomerism of the amide–imidol type.

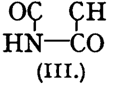
Tautomerism of Barbituric Acid.—The triketonic form of the acid (I) can give three monoenolic forms (II, III, and IV) and five dienolic forms. The trienolic form (V) would predominate in alkaline solution.



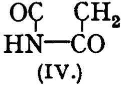
(I.)



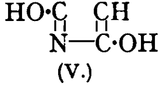
(II.)



(III.)



(IV.)



(V.)

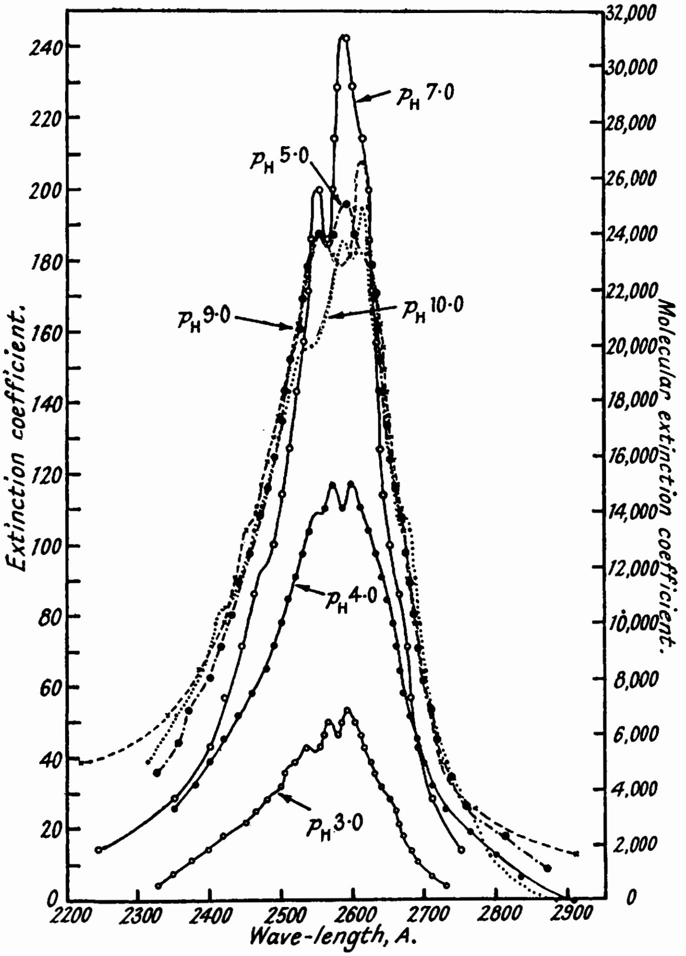
Experimental.

Barbituric acid (Eastman) was thrice recrystallised from aqueous alcohol. Glass-distilled water, preserved in Pyrex flasks, was used for all solutions. Kolthoff buffers were employed for adjusting the p_H, solutions of the buffers alone, at appropriate concentrations, being used in the comparison cell in determining spectra. The concentration used for spectra was 7·7 mg.

per ml. (2-cm. cells). Details of the spectrographic technique, methods of irradiation, etc., were published in Part I (*loc. cit.*).

Figs. 1 and 2 show the influence of p_{H} on the barbituric acid absorption spectrum. [The extinction coefficient is given by $(1/cd)\log_{10} I_0/I_x$, with c in g./l. and d in cm.] At p_{H} 7, there is a sharp maximum at 2590 A. with a molecular extinction of 31,000. Unbuffered solutions at the concentrations employed had a p_{H} of approximately 5. At p_{H} 5·0, we found a molecular extinction of 26,000, which compares with Castille and Ruppol's value of 20,000 and Heyroth and Loofbourow's of 25,000 for unbuffered solutions.

Fig. 1.



Maximum extinction occurred at p_{H} 7·0, change of p_{H} towards the acid side resulting in more marked decrease in absorption than change towards the alkaline side. No doubt, Macbeth, Nunnan, and Traill (*loc. cit.*) failed to note this effect of p_{H} because they examined only the unbuffered aqueous solution and one containing N/10-sodium hydroxide, thus, by accident, choosing two curves on opposite sides of the p_{H} 7 maximum, for which the extinction would be expected (from our data) to be approximately the same.

The maximum on our curves is resolved into two or three subsidiary peaks which may well correspond to the contributions to the absorption of individual unsaturated groups in the molecule. The narrowness and steepness of the curves for p_{H}'s near 7 are presumably caused by the absorptions contributed by each of the absorbing groups all having their maxima at approximately the same wave-length. This is borne out by the fact that the subsidiary peaks on all the curves lie approximately within the limited range 2550—2600 A. If one can assume that at the most acid p_{H} the molecule is in the form (I), and at the most alkaline p_{H} in the form (V), this implies that in barbituric acid the influence of substituent groups, etc., is such that all possible $C=O$, $C=N$, and $C=C$ groups, in the various tautomeric forms, contribute absorption at approximately the same wave-length. Since the minimum absorption occurs at the most acid p_{H}, the contribution of $C=O$ groups to the absorption is apparently less than that of $C=N$ or $C=C$ groups.

The explanation of the occurrence of greatest absorption at p_{H} 7 may lie in the many theoretically possible resonating forms of this compound in the intermediate p_{H} range.

FIG. 2.

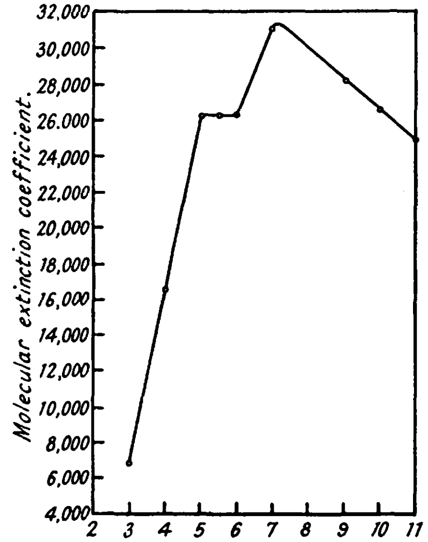
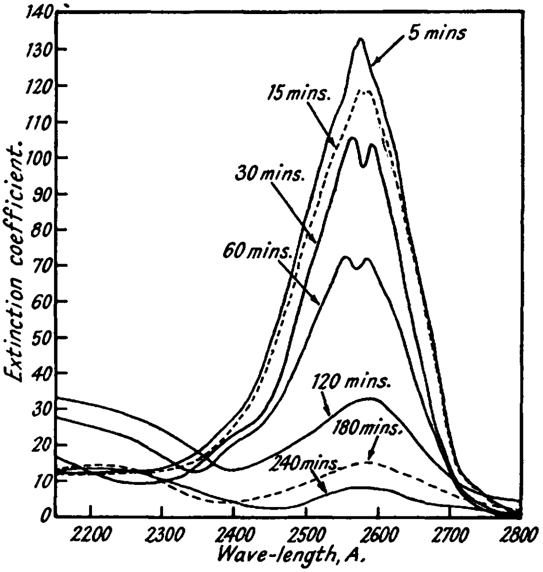


FIG. 3.



In addition to the molecular rearrangements discussed, salt formation in alkaline solution and addition of hydrogen chloride to the nitrogen atoms in acid solution must be considered as possible effects contributing to the influence of p_{H} on absorption. Further discussion of the various factors influencing absorption in relation to p_{H} is deferred until data for the remaining compounds in the series are obtained.

Ultra-violet irradiation of barbituric acid in unbuffered solution at p_{H} 4·6 resulted in marked progressive decrease in extinction (see Fig. 3). This is in contrast to the negligible change found under the same conditions for adenine (this vol., p. 848). As will appear from the subsequent papers, lability to ultra-violet irradiation in this series of compounds appears to parallel closely the number of carbonyl groups in the molecule.

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